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Laser Doppler Perfusion Imaging: New Technique for Determination of Perfusion and Reperfusion of Splanchnic Organs and Tumor Tissue

David L. Liu, MD, PhD, Katarina Svanberg, MD, PhD,* Ingrid Wang, MD, Stefan Andersson-Engels, PhD, and Sune Svanberg, PhD, MDhc

Department of Oncology, Lund University Hospital, Department of Physics, Lund Institute of Technology, and the Lund University Medical Laser Center, Lund University, S-221 85 Lund, Sweden

Background and Objective: **Several investigations indicated that laser Doppler flowmetry on the liver surface reflects relative changes of the total liver blood flow. In this study, Laser Doppler Perfusion Imaging (LDI), monitoring the surface only, was used for measurements of tissue perfusion of normal and/or impaired liver, pancreas, spleen, stomach and intestine, and the blood flow of hepatic tumors in rats.**
Study Design/Materials and Methods: **Eighty Wistar/Furth rats were divided into five groups. Group I served as controls. Groups II and III underwent ischemic injury of the liver and intestine with or without the administration of WEB2170, a platelet-activating factor receptor antagonist. Laser-induced photodynamic therapy (PDT) utilizing δ-amino levulinic acid sensitization was performed in Groups IV and V.**
Results: **Normal pancreas and intestine had a high LDI perfusion value and the liver and stomach exhibited a medium perfusion value whereas the perfusion value from the spleen was low. WEB 2170 improved the reperfusion of the postischemic liver and intestine. An immediate decrease in surface blood flow of hepatic tissue treated by laser-induced PDT and a decreased blood flow in large tumors were observed.**
Conclusion: **LDI is a useful technique for the measurement of tissue perfusion of various splanchnic organs or tumor tissues.**
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Key words: laser; laser Doppler imaging; liver; microcirculation; neoplasm; perfusion; tumor

INTRODUCTION

Determination of tissue perfusion of organs and tissues is of considerable importance in evaluating physiological functions of the body and pathological mechanisms in diseased organs or tissues. It also has a great clinical value in planning treatment schedules in patients with various ischemic diseases or microcirculatory disorders.

Several techniques can be applied to the measurement of tissue perfusion, including the use of radiolabelled microspheres, Xenon-133 clearance, intravital microscopy, duplex ultrasound systems, and hydrogen gas clearance test [1–5]. However, these methods appear to have their limitations, e.g., inhomogeneous distribution of the radiolabelled microspheres in tissue, damage to tissues or organs to be examined, or technical problems.

Laser Doppler Flowmetry (LDF) was devel-

*Correspondence to: Katarina Svanberg, M.D., PhD, Department of Oncology, Lund University Hospital, S-221 85 Lund, Sweden.

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oped in the late 1970s as a method to measure tissue perfusion [6]. During the last decade, LDF has gained widespread use both experimentally and clinically as a continuous, real-time, noninvasive technique for tissue perfusion measurements. Previously, the application of LDF in the determination of the blood flow of normal and ischemic liver was presented [7,8]. However, the LDF point-monitoring method exhibits obvious drawbacks as the value of the tissue perfusion is easily influenced by the pressure of the probe fiber tip at the tissue measured [8,9].

Laser Doppler Imaging (LDI) is a noncontact laser imaging technique for the measurement of tissue perfusion. Wårdell et al. [10] and Svanberg et al. [11] have reported on the use of LDI in evaluating the tissue perfusion of the skin, intestinal mucous, and cutaneous malignancies. The present study was performed in order to evaluate the use of LDI in the connection of tissue perfusion of normal liver, pancreas, spleen, intestine, and stomach, in combination with blood flow occlusion for the induction of tissue ischemia. In addition, the role of WEB 2170, a platelet-activating factor receptor antagonist, in improving the reperfusion of the postischemic liver and intestine was evaluated. Furthermore, LDI was used for the monitoring of the tissue perfusion in an experimental liver tumor treated with laser-induced photodynamic therapy (PDT) utilizing δ-amino levulinic acid (ALA) sensitization.

MATERIALS AND METHODS

Animals

Eighty Wistar Furth (W/Fu), rats weighing 180–220 g, were divided into five experimental groups. Group I (10 rats) served as a control group, and the tissue perfusion of normal liver, pancreas, spleen, stomach, and intestine was monitored. Group II (20 rats) underwent warm ischemia of the liver and gut. The liver ischemia was induced by an occlusion of the portal triad (the hepatic artery, portal vein, and common bile duct) and the intestinal ischemia by occlusion of the celiac artery. The duration of the occlusion was 90 min. The tissue perfusion of the ischemic liver and gut was measured before the block of the portal triad and interruption of the celiac artery. The measurements were repeated 60 min after the release of the portal triad and the celiac artery. The animals in Group III (20 rats) were treated with WEB 2170 (Boehringer Ingelheim KG, Germany). WEB 2170 was intravenously administered at the dose of 6 mg/kg of body weight 5 min before the start of the occlusion procedure. The tissue perfusion in this group was determined as in Group II. Normal livers of the animals in Group IV (10 rats) were treated with laser-induced PDT, and the blood flow in the irradiated normal liver was measured before and immediately after the laser irradiation procedure. Group V (20 rats) was investigated 7 days after the inoculation of a hepatic tumor. The model of liver tumor was induced by the inoculation of 3×10^5 viable tumor cells into the subcapsular area of the left liver lobe [12]. The tumor size was 6–7 mm in diameter at the time for the investigation.

Surgical Procedure

A general anesthesia was performed with ether. All surgical procedures were carried out under clean conditions. A fine silicone cannula was placed in the jugular vein of the rat for continuous volume replacement with Ringer-glucose solution (0.3 ml/kg/min) or for administration of drugs using an injectomat (Fresenius AG, Bad Homburg, Germany). The abdomen was opened through a midline incision. The xiphoid process of the rat was vertically hanged over a special shelf, and four small tractors were set up to expose the liver, stomach, intestine, pancreas, and spleen.

The triangular ligaments and falciform ligament of the liver were divided and two to three cotton swabs were placed under the diaphragmatic membrane in order to pull the mediate and left liver lobes down for the measurement of blood flow in the liver or liver tumor(s). The spleen and pancreatic tail were gently elevated and moved to the middle part of the abdominal cavity. The stomach and intestine were freely mobilized where they were measured. Abdominal organs not investigated were covered by gauze soaked with normal saline to prevent them from the loss of water throughout the experimental course.

Laser-induced PDT

All animals in Groups IV and V received ALA (Porphyrin Products, Logan, UT) intravenously at a dose of 60 mg/kg of body weight 60 min before the laser irradiation. A total light dose of 100 J/cm^2 was delivered. A high repetition-rate Q-switched Nd:YAG laser system (Multilase 2500, Technomed International, Bron, Paris, France), frequency doubled to 532 nm, was used to pump a dye laser tuned to 635 nm. The light power density was kept below 110 mW/cm^2 in order to avoid local hyperthermia. The laser light

was delivered through a 600 μm quartz fiber. The polished end face of the fiber was imaged using a 40× microscope objective onto the surface of normal liver and hepatic tumor, so that the area was irradiated by a uniform "top hat" light intensity distribution. Without such an arrangement, the laser light intensity would be higher in the center, with a gradual fall-off in light intensity toward the borders of the treated area.

Monitoring of Arterial Blood Pressure

A Portex cannula (Size 3FG, Portex, Hythe, UK) was placed in the femoral artery of the animal. The cannula connected to a hemodynamometer (Mingograf 7, Siemens, Erlangen, Germany) for monitoring of the systemic arterial pressure in Groups II and III, intraoperatively.

Laser Doppler Perfusion Imaging of Visceral Organs

The laser Doppler perfusion imager (Lisca Development AB, Linkoping, Sweden) is constructed to be used noninvasively to produce tissue perfusion images. The instrument consists of a laser scanner with a detector and a signal processor, a computer, and a color plotter. The operating principles of this instrument have been described previously [10]. A light beam from a 3 mW He-Ne laser is reflected onto the tissue by an optical mirror system. The 0.8 mm diameter light beam is moved step by step over the object, penetrating the tissue to a depth of a few hundred microns. In the presence of moving blood cells, a fraction of the backscattered and Doppler-broadened light is collected by a photodetector and is converted into an electrical signal. The latter is further processed to scale linearly with the blood flow that is eventually used as an estimator of the tissue perfusion defined as the product of the blood cell velocity and the concentration in the sampling volume. The system is assisted with an IBM-compatible PC. Using software it is possible to display the perfusion values along a line over the image. Further, basic statistical tools are available for image analysis. The LDI image of tissue perfusion can be automatically recorded, stored, re-displayed, and printed.

The recording conditions for the LDI were standardized during the measurements of blood flow of the splanchnic organs in this study. During all measurements, the distance between the laser scanner head and the tissue measured was fixed to 12 or 15 cm. The recorded signals were displayed with false-color coding, but also could be converted to a gray scale. The same instrumental electronic settings were used for all measurements. Signals below a certain threshold were displayed as the background. Maximal signal was represented with 100% tissue perfusion and no signal with 0%.

Statistical Analysis

A statistical analysis of the pixel values of blood perfusion (mean value and standard deviation) within selected areas of a laser Doppler image can be performed with the software provided. However, the average of the mean values for a group of animals is more interesting.

Data from this study are thus presented for the individual groups and expressed as mean values. The groups were compared by analysis of variance and Student's t-test using a commercially available statistical program. A $P < 0.05$ was considered statistically significant and $P < 0.01$, highly significant.

RESULTS

Changes in Systemic Arterial Pressure

With time of occlusion of the portal triad, a progressive decrease in arterial pressure was observed in Groups II and III. LDI perfusion values reflected the decrease in arterial pressure in a progressive hypo-perfusion of the ischemic liver and intestine.

Perfusion of Normal Liver, Jejunum, Pancreas, Spleen, and Stomach

High Doppler signals were recorded from normal pancreas (Fig. 1) and intestine (Fig. 2). The mean values of LDI perfusion were 82 ± 6% for the pancreas and 76 ± 8% for the intestine, respectively. The liver and stomach showed an intermediate blood perfusion with the mean values of 48 ± 8% and 40 ± 10%, respectively. In contrast, the spleen showed an extremely low blood perfusion with a tissue perfusion mean value of 16 ± 6% (Fig. 1).

Reperfusion of Ischemic Liver and Gut

During the occlusion of the portal triad or the celiac artery, markedly reduced reperfusion values of the ischemic livers and intestines were seen, which was consistent with reduced systemic arterial pressure values. A mean perfusion value of 16% in the ischemic livers and 14% in the ischemic gut was detected in Group II.

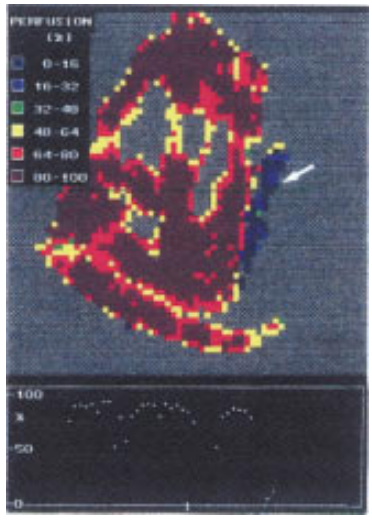

Fig. 1.

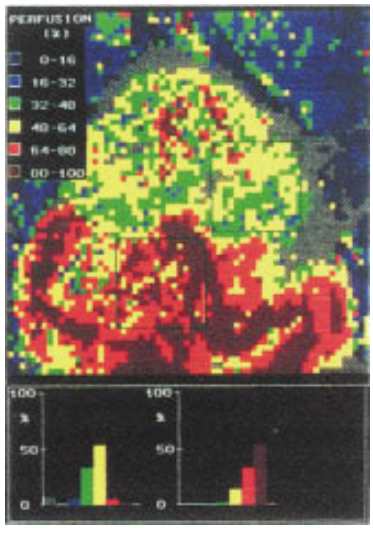

Fig. 2.

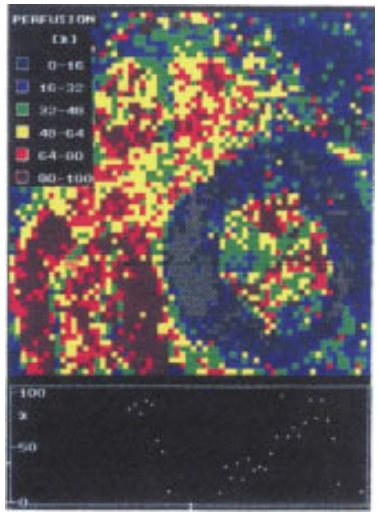

Fig. 3.

Effect of WEB 2170 on Postischemic Liver and Intestine

An improved tissue perfusion of the ischemic liver and gut was found in animals treated with WEB 2170. The mean LDI value in Group III was for the liver 41 ± 8% and the intestine 70 ± 10%, respectively. There was a statistical significance ($P < 0.05$) in the perfusion value (the ischemic liver and intestine) of LDI between preischemia and postischemia.

Tissue Perfusion of Hepatic Tissue Undergoing ALA-PDT

Immediate decline in blood flow was found in the PDT treated liver tissue (Figs. 3, 4). The

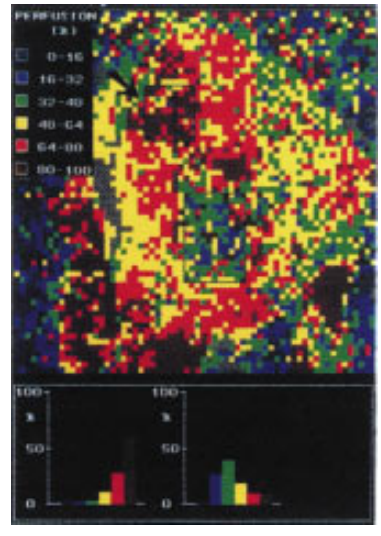

Fig. 5.

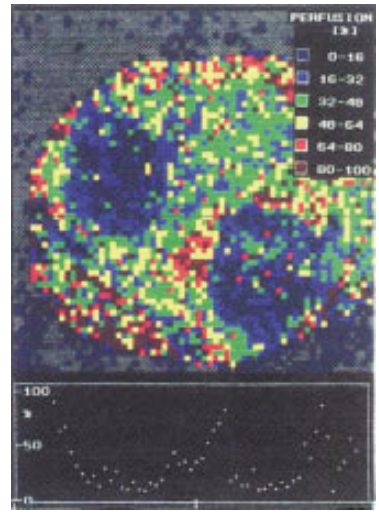

Fig. 6.

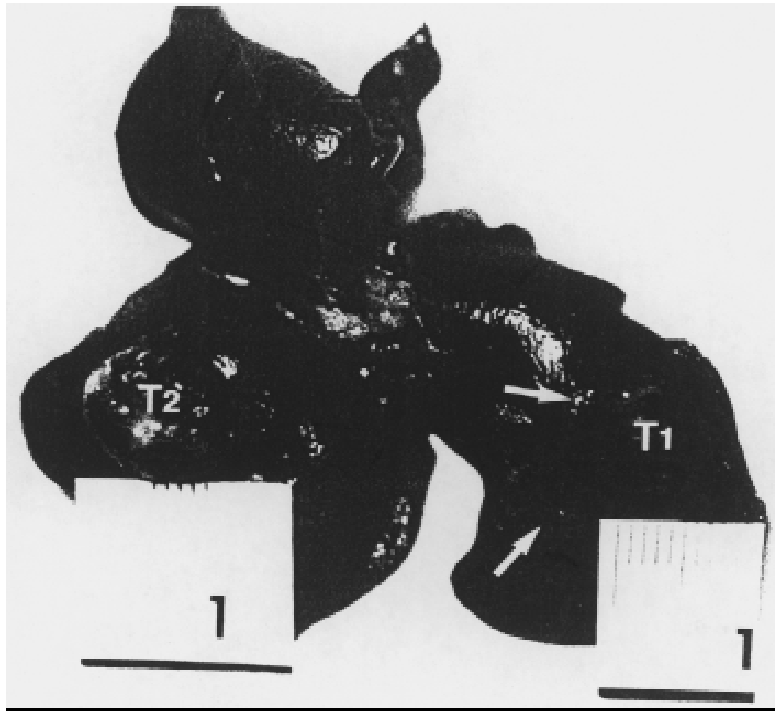

Fig. 4. Photograph showing a liver tumor with a PDT-treated area (arrows, T1). Another nontreated tumor (T2) is seen on the right lobe of the liver.

perfusion value of liver tissue decreased to the posttreatment level of 16 ± 6% from a pretreatment level of 46 ± 8% ($P < 0.05$).

Perfusion of Liver Tumor

The perfusion values for liver tumor were related to tumor size. When the diameter of the tumors was <7 mm, blood perfusion with an LDI value of 56 ± 8% was recorded (Fig. 5). A lower perfusion at the center of liver tumors was observed in the cases of a tumor diameter >10 mm (mean perfusion value 25 ± 10%) (Fig. 5), due to the development of a central necrosis. The perfusion value (55 ± 10%) of the peripheral zone of all liver tumors was higher than the value for normal liver tissue (Fig. 6).

Fig. 1. LDI image of normal pancreas and spleen in a rat. The perfusion values in the image are given on a false color scale. Values along a line across the image are displayed in the lower part of the figure. The reddish area in the center of the figure represents pancreatic tissue that exhibits a high tissue perfusion. The spleen, marked with an arrow in the right part of the figure, shows a low perfusion (blue).

Fig. 2. An LDI image with statistical bar charts from areas on normal rat liver and intestine. The distribution of pixel values within the selected square areas are shown. The left liver lobe (the upper marked area) displays a medium level of blood perfusion (49 ± 10%; high occurrence of medium intensity pixels; left diagram) and the intestine (the lower marked area) shows a high blood perfusion (77 ± 12%, high occurrence of high intensity pixels; right diagram).

Fig. 3. The value of the surface blood perfusion in the surrounding tissue (circular zone) of the tumor reduces immediately following PDT (18 ± 5%). Values along a line through the tumor are displayed in the lower part of the figure. The reduction in blood flow may explain the mechanisms of PDT in the treatment of experimental liver tumors.

Fig. 5. A small liver tumor (arrow) is seen on the right upper quadrant showing a high perfusion value (78 ± 18%: left diagram). In contrast, a larger liver tumor positioned on the liver left lobe shows a low perfusion (45 ± 20%; right diagram).

Fig. 6. The perfusion profile from a rat liver tumor before PDT. Two liver tumors are seen in the right and left liver lobes, respectively. Both tumors are large with central necrosis. The necrotic areas show a low perfusion value. Conversely, high perfusion values of the peripheral tissue of both liver tumors are observed.

DISCUSSION

The application of a point-monitoring laser Doppler flowmeter (LDF) in determination of tissue perfusion was described in 1980 by Nilsson et al. [6]. The technique has been used in animal experimental investigations and in studies of patients with impaired microcirculation of organs or tissues. Recently, LDF has been used in the assessment of blood perfusion of the skin, liver, stomach, intestine, spinal cord, and cerebral tissue [7,8,13,14].

LDF has been used to monitor the perfusion of normal liver tissue and reperfusion of the postischemic liver in rats [8]. However, LDF values may be influenced by, e.g., the pressure of the probe fiber tip on the tissue examined in contact measurements.

The laser Doppler imaging system used in this study has several advantages as compared to the point-monitoring LDF: (1) the LDF measurements take place with the probe fiber in contact with the tissue investigated; the LDI monitors the blood flow in a noncontact mode, allowing for more accurate evaluation of the tissue perfusion, (2) the instrument is assisted with a statistical analysis menu that makes it possible rapidly to perform basic data analysis, and (3) the result of the tissue perfusion is presented as a false-color image, a perfusion profile along a selected line through the image or a barchart for the pixels within selected areas. This is valuable in both experimental and clinical studies. Therefore, LDI can be regarded as the second generation of the blood flow measurement instruments utilizing laser Doppler technology.

It is worth noting that LDI can be used for hemodynamic observations of normal tissues. The data from the present study demonstrate that the perfusion values of ischemic livers and intestines progressively decrease with the prolongation of the occlusion time of the portal triad and celiac artery. This is in agreement with the result observed by monitoring the systemic arterial pressure.

By means of LDI, we found that the platelet activating factor receptor antagonist, WEB 2170, effectively improved the reperfusion of the postischemic liver and intestine, but no effects were found in normal liver or gut. We also noted that the hepatic tissue treated with ALA-PDT exhibited an immediate hypo-perfusion phenomenon, indicating that tissue perfusion in the treated liver tissue dramatically decreased following PDT. Thus, LDI may be a useful method for evaluating the treatment procedure in tumor-bearing rats.

LDI in the examination of tissue perfusion of rat liver tumors showed a strikingly high blood perfusion in small liver tumors. In contrast, the perfusion values in larger liver tumors showed a low value in the central part of the tumors. Most probably this can be explained by necrosis formation in the central part of a tumor. Another interesting finding from the present study is that the peripheral tissue of large liver tumors showed a high perfusion even in cases with a central necrosis. This is in agreement with the report by Rubin et al. [15]. These findings are of great clinical value in the planning strategies for the treatment of patients with liver tumors.

LDI instrumentation is designed for superficial tissue perfusion monitoring and can be used as shown in the present study in the measurement of blood perfusion of the visceral organs intraoperatively. Arvidsson et al. [16] reported that LDF on the liver surface reflected relative changes of the total liver blood flow. Our previous study using LDF [7] also showed that there was no significant differences in blood perfusion between liver surface and hepatic parenchyma.

The present preliminary study shows that LDI can be a valuable tool in the investigation of the tissue perfusion of the abdominal and thoracic organs during a surgical procedure, e.g., in the examination of the perfusion statue of a transplanted organ such as the heart, liver, or kidney or in patients who have a bypass operation following surgery. LDI has been applied in dynamic observation of the blood flow of skin tumors before and after PDT with the application of topical ALA [11].

The application of LDI in the observation of tissue perfusion of diseased organs may lead to better understanding of the pathogenesis. It is well known that there is a relationship between tissue vitality and tissue perfusion. LDI may be especially useful in intraoperative diagnosis of a strangulated hernia or a necrotic intestinal obstruction. This might guide the surgeon in judgments intraoperatively, whether, e.g., an intestinal loop has lost its vitality or not.

However, it should be noted that flow values determined by the laser Doppler technique are influenced by tissue optical properties. Therefore, a direct comparison of blood perfusion between various tissue types using this method is not possible, whereas blood perfusion values from the same type of tissue can be interrelated.

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